

**2. [Original]** The method of claim 1, wherein the chemical reagent is a carbodiimide.

**3. [Original]** The method of claim 1, wherein the chemical reagent forms amide bonds.

**4. [Original]** A method of enzymatically degrading cotton fibers to yield essentially pure cellulose comprising the steps of sequentially treating the fibers first with cellulase and then with protease.

**5. [Original]** A method of characterizing cotton fiber cell walls comprising the steps of specific enzyme degradation in sequential steps utilizing cellulases and proteases.

**6. [Original]** The method of claim 5, wherein the cellulases are utilized at different pH's to accentuate differences between cotton fibers of different varieties.

**7. [Original]** The method of claim 5, wherein different types of proteases are utilized.

8. **[Original]** The method of claim 7, wherein the different types of proteases are utilized sequentially.

9. **[Original]** The method of claim 5, wherein different types of cellulases are utilized.

10. **[Original]** The method of claim 9, wherein different types of cellulases are utilized sequentially.

11. **[Currently Ammended]** The methods of **any of the** claims 5-10, further comprising the step of utilizing the characterization of cotton fibers according to the methods to develop biochemical markers for fibers of different cotton ~~varieties~~ **cultivars**.

12. **[Currently Ammended]** The method of **any of the** claims **5-10**~~44~~, wherein the biochemical markers are used in plant breeding to improve fiber quality.

13. **[Currently Ammended]** The method of **any of the** claims **5-10** ~~44~~, wherein the biochemical markers are used as a means to distinguish ~~varieties~~ **cultivars** of cotton.

1. With respect to claims 1-3, the extrapolation from Murray(WO 99/35491) to the present claims is very tenuous at best. We know the glycan oligomers contain glucose most likely in a  $\beta$ -1,4 linkage due to the degradation by endo- $\beta$ -1,4-glucanase. The assumption of Murray is that these oligomers contain one

linear chain. However, more recent work indicates that is probably not the case. That is to say that there are probably several shorter chains rather than one long chain based on the solubility of the oligomers. The assumption of Murray that these oligomers are linked to protein is also probably erroneous as the data presented there only demonstrates co-chromatography of the oligomers with some substance which absorbs at 280nm. It should also be pointed out that the carbodiimide cross-linking procedure of claims 1-3 has been in the parent applications of the current application but the examiners felt the subject matter was too broad and requested that these claims be included in a subsequent continuation-in-part application.

2. With respect to claim 4, Murray(WO 99/35491)(P23/L10-11) only states that fibers can be degraded using a cellulase followed by a protease but does not mention it in the context of the present patent application for the purposes indicated in the title of this present patent application. Further, Murray makes no mention of the characterization based on the variety of intermediates that have been released as shown in Figures 14 and 15 or the differential seen in insoluble hydrolysis products as shown in Figures 17 and 18. This result was not obvious to me or anyone else. In fact, the description of intermediate hydrolysis products shown in Figures 14 and 15 has not been demonstrated in any publication other than this application and the same is true for the hydrolysis products shown in Figures 17 and 18. I argue strongly that due to this unexpected result the "obviousness" argument does not hold up.

3. With respect to claims 5 and 7-10, I would only like to reemphasize my response from the previous Office Action:

*The impression that with respect to claims 5-10, Murray teaches that enzymatic degradation of 25DPA cotton fibers using the steps of a cellulase followed by a protease and that the products resulting from said degradation such as glycoconjugates, which are disclosed as cell wall precursors, can be analyzed (P8/L6-23) is simply not true. The glycoconjugates being discussed in Murray (P8/L6-23) are those extracted with cold water which are presumed to be cell wall precursors based on their appearance and disappearance with different stages of development. To assume that any glycoconjugate released by enzymatic or chemical means is also a cell wall precursor is an erroneous assumption. Clearly, any constituent released from the fiber by chemical or enzymatic means is by definition a "cell wall constituent" or a "cell wall component" but by no stretch of the imagination could it be called a cell wall precursor because it was released from an already formed cell wall. Further, Murray (P22/L20) **DOES NOT** discuss pH levels with respect to fiber degradation. The pH 5.0-5.2 mentioned in Murray (P22/L20) refers to the unbuffered pH of the reaction mixture with carbodiimide*

4. With all due respect, the material included in Clarkson, et. al., does not permit one to make the extension to the obviousness argument for the present claims. To begin with, the intent of the work of Clarkson, et. al. has nothing to do with the characterization of the cell wall but rather it deals only with finished fabric and it is only to use enzymatic means to replace the stones used in stone

washed fabric and to use proteases to prevent the blue indigo dye from backstaining the cellulase treated fabric. It should be pointed out that during the textile production and finishing process the fabric is exposed to several harsh chemical treatments and the primary cell wall has been removed. In the present work, I am using raw cotton fibers removed from the boll and immature fibers (21DPA and 44DPA) at that. Commercial fabric is only made from mature fibers (56DPA and older). There is no way a direct correlation can be made between immature fibers and mature fibers after they have been incorporated into a finished textile product for many of the conclusions from the present work.

Again, with all due respect, I seriously object to the statement: "The level of abrasion and reflectance would be a characterization of the fiber cell wall." I have been involved in plant cell wall research since 1968 and I have never heard of "abrasion" or "reflectance" being used to characterize any plant cell wall. I can't imagine how such measurements would be feasible except with a piece of woven fabric because they could not be done on a single cell or random group of cells in a test tube. In addition, I know from personal conversations with Clarkson and colleagues that they do not use and would not even consider using a purified enzyme to treat fabric due to the cost of purification on a commercial scale. They only use a filtered culture broth of whatever fungus they are dealing with. So any cellulose or protease used in the work of Clarkson, et. al., is a crude enzyme in a culture broth which obviously contains many other components including other enzymes. While the cellulases and proteases used in the present work are highly purified enzymes. It is not possible to extrapolate